b) applying an electric field to said sample, wherein said phosphate detectable moiety migrates to an electrode differently than said intact charge-switch NP probe.

IN THE DRAWINGS:

Applicants submit herewith proposed amended drawing for Figures 4, 6E, 7, 9 and 10, for the Examiner's consideration. The details of the amendments are set forth below.

REMARKS

Claims 1-14 and 16-54 are pending in this application and are presented for examination. Claims 1, 6 and 18 have been amended. Claim 15 has been canceled without prejudice.

Support for the amendments to claims 1 and 18 is found, *inter alia*, in claim 5 as originally filed and on page 15, lines 11-12, respectively. No new matter has been introduced with the foregoing amendments. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made." For the Examiner's convenience, the pending claims are attached. Reconsideration is respectfully requested.

I. RESTRICTION REQUIREMENT

Applicants maintain their traversal of the restriction requirement set forth in Paper No. 10. Applicants elected Group II, drawn to a method for identifying an intact charge-switch NP probe **and** to an intact charge-switch NP probe, with traverse. Claims readable thereon included claims 1-44. The reasoning behind such election was to take advantage of M.P.E.P § 821.04, wherein, if Applicants elect claims directed to the product (the intact probe), and the product claims are allowable, withdrawn process

claims which depend from or otherwise include all the limitations of the allowable product claims must be rejoined. Rejoinder is a matter of right.

However, in the Office Action dated May 21, 2002, the Examiner did not examine the subject matter elected by Applicants drawn to the products (claims 29-44). As the Examiner has examined the method claims of 1-17 and 18-28, Applicants respectfully request that the other method claims of 45-54 be rejoined, and examined on their merits.

II. FORMALITIES

With regard to the Examiner's allegation that the declaration is defective, please note that the present application was filed with an Application Data Sheet (ADS) pursuant to 37 CFR § 1.76. The ADS filed with the present application provides the full mailing address for each inventor. As such, Applicants believe the declaration as filed is proper and therefore, respectfully request that the Examiner withdraw the objection to the declaration.

III. DRAWINGS

Applicants submit herewith proposed amended drawings for the Examiner's consideration.

The Examiner objected to Figure 4 as the description on Page 4, line 16, states that BODIPY is conjugated to dTTP whereas the figure legend states the structure is dTTP-BQS-BTR. In response, the figure legend on page 7, line 3, has been corrected. Further, a proposed substitute Figure 4 has been submitted herewith. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection.

The Examiner objected to Figure 6 as allegedly the details of the synthesis processes described in the specification are not shown. In response, Applicants submit herewith a substitute Figure 6E, with larger print. No new matter has been introduced,

however, with the larger structures, the details are more clearly illustrated. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection.

The Examiner objected to Figure 7 as allegedly there is no reference in the description for "73". Applicants submit herewith, a proposed substitute Figure 7 with "73" removed. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection.

The Examiner objected to Figure 9 as allegedly there is no reference sign in the description for "97". Applicants submit herewith, a proposed substitute Figure 9 with "97" removed. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection.

The Examiner objected to Figure 10 as allegedly there is no reference sign in the description for "1000, 1072(a) and 1072(b)". Applicants submit herewith, a proposed substitute Figure 10 wherein the numbers are commensurate with the description. In view of the foregoing, Applicants respectfully request that the Examiner withdraw the objection.

IV. REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 1-28 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that which Applicants regard as their invention. In response, each of the Examiner's concerns, and Applicants' response to those concerns, will be addressed in turn.

The Examiner has rejected claim 1, alleging that the term "NP" is not defined. In response, as set forth throughout the specification as filed, "NP probe" stand for nucleotide phosphate probe (for example, please see page 3, line 15). The term nucleotide is defined on page 8, lines 26-33, bridging to page 9, lines 1-2. As such, Applicants believe NP probe is clearly defined and respectfully request that the Examiner withdraw this rejection.

In addition, claim 1 was rejected as allegedly being unclear. The Examiner states, "if the probe is cleaved, it is no longer intact." In response, Applicants respectfully traverse the rejection.

Amended claim 1 sets forth:

> A method for separating an intact NP probe from a phosphate detectable moiety, said method comprising:
>
> a) providing a sample comprising an intact NP probe with a detectable moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe to incorporate said NP probe on a primer strand hybridized to a target nucleic acid, a phosphate detectable moiety is produced, wherein said phosphate detectable moiety carries a molecular charge which is different than the molecular charge of said intact NP probe; and
>
> b) applying an energy field to said sample, thereby separating said phosphate detectable moiety from said intact NP probe.

A reading of the claim makes clear that the energy field is separating an intact probe from a phosphate detectable moiety. The fact that an intact probe is cleaved is of no moment to other "intact NP probe(s)" in the sample. Clearly, upon reading the claim in view of the specification, a skilled artisan would be cognizant that more than one NP probe is present in the sample. In view of the foregoing amendment and remarks, the claim is clear and definite, and Applicants respectfully request that the Examiner withdraw the rejection.

The Examiner alleges improper antecedent basis in claim 3, which recites "said charge-switch nucleotide phosphate" in lines 1 and 2. However, claim 3 is dependent on claim 2, wherein proper antecedent basis is found. As such, Applicants respectfully request that the Examiner withdraw this rejection.

The Examiner has rejected claim 4, alleging that "it is not clear into what the probe is incorporated and how." To the extent the rejection is applicable to the

amended set of claims, Applicants respectfully traverse the rejection. Claims 3 and 4 recite:

> 3. The method according to claim 2, wherein said charge-switch nucleotide phosphate is a nucleotide triphosphate (NTP) having a γ-phosphate with a detectable moiety attached thereto.
>
> 4. The method according to claim 3, wherein said γ-phosphate with a detectable moiety attached thereto is a γ-phosphate with a fluorophore attached thereto.

As amended, Claim 1 make clear that the NP probe is incorporated on a primer strand hybridized to a target nucleic acid, wherein a phosphate detectable moiety is produced. Further, claim 4 describes the γ-phosphate with "a detectable moiety" as being a γ-phosphate with "a fluorophore." Although not intending to be limiting, claim 4 reads on compounds of Formula I at the top of page 15, of the specification as filed. As such, Applicants respectfully request that the Examiner withdraw this rejection.

The Examiner has rejected claim 6, alleging improper antecedent basis. In response, Applicants have amended claim 6 to properly depend from claim 5. In view of the foregoing amendment, Applicants respectfully request that the Examiner withdraw this rejection.

V. DOUBLE PATENTING

Claims 1-28 were rejected under the judicially created doctrine of double patenting as allegedly being obvious over claims 22-36 of co-pending U.S. Patent Application No. 09/876,374. Applicants respectfully request that this rejection be held in abeyance until the present application is found allowable. At that time, Applicants will take the necessary steps to obviate the double patenting rejection.

VI. REJECTION UNDER 35 U.S.C. § 102(e)

The Examiner rejected claims 1-4, 7, 10-14, and 16-28 under 35 U.S.C § 102(e) as allegedly being anticipated by U.S. Patent No. 6,323,186 ("Klaubert *et al.*"). The Examiner alleges that Klaubert teach a nucleotide with a fluorescent labeled bound to the phosphate moiety wherein the nucleotide can be a mono-, di-, or triphosphate. The labeled nucleotides are allegedly used to generate a fluorescent phosphate moiety.

To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

> To anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter. (*see, PPG Industries Inc. v. Guardian Industries Corp.*, 37 USPQ2d 1618, 1624 (Fed. Cir. 1996)).

Claim 1 as amended sets forth *inter alia*, a method for separating an intact NP probe from a phosphate detectable moiety, comprising: providing a sample comprising an intact NP probe with a detectable moiety attached thereto, whereupon an enzymatic cleavage of the intact NP probe to incorporate the NP probe on a primer strand hybridized to a target nucleic acid, a phosphate detectable moiety is produced, wherein the phosphate detectable moiety carries a molecular charge which is different than the molecular charge of the intact NP probe.

Klaubert *et al.* do not teach or suggest the use of an intact NP probe to incorporate the NP probe on a primer strand hybridized to a target nucleic acid, as is presently taught and claimed. The phrase "target nucleic acid" refers to for example, a nucleic acid or a polynucleotide whose sequence identity or ordering or location of nucleosides and the like, is to be determined using certain of the methods described herein.

In the present method, an intact NP probe is separated from the phosphate detectable moiety. The method includes providing a sample comprising an intact NP probe wherein the NP probe is incorporated on a primer strand hybridized to a target

nucleic acid. After enzymatic cleavage of the NP probe, a phosphate detectable moiety is produced. As the phosphate detectable moiety carries a molecular charge different than the molecular charge of the intact NP probe, the phosphate detectable moiety can be separated form the intact NP probe by applying an energy field. As Klaubert *et al.* do not teach or suggest the use of a target nucleic acid, the present methods are not anticipated.

Further, claim 18 has been amended to recite that the intact charge-switch NP probe has a charged moiety on the base. Klaubert *et al.* in no way teach or suggest the use of a nucleoside base having a charged moiety. As such, Klaubert *et al.* do not anticipate the claims. Therefore, Applicants respectfully request that the Examiner withdraw the rejection.

VII. REJECTION UNDER 35 U.S.C. § 103(a)

The Examiner rejected claim 15 under 35 U.S.C. § 103(a) as allegedly being obvious over Klaubert *et al.* in view of 5,814,524 ("Walt *et al.*"). The Examiner alleges that it would have been obvious to use the optical fiber sensor of Walt in the phosphate detection method of Klaubert. To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

Applicants have canceled claim 15 rendering this rejection moot. A such, Applicant respectfully requests that the Examiner withdraw the rejection.

VIII. CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

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WC 9044866 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the specification on page 1, line 1 as follows:

This application claims priority to U.S. Provisional Patent Application Nos. 60/209,896, filed June 7, 2000, and 60/286,238, filed April 24, 2001, both the disclosures of which are hereby incorporated by reference in their entirety for all purposes. This application is related to U.S. Patent Application No. 09/876,374 **[bearing Attorney Docket Number]** filed on even date herewith, which is hereby incorporated by reference in its entirety for all purposes.

Please amend the specification on page 7, line 1 as follows:

FIG. 4 illustrates a compound of the present invention (dTTP-BQS(++)-BODIPYTR) **[(dTTP-BQS-BTR)]**;

Please amend claims 1, 6 and 18 as follows:

1. (Amended) A method for separating an intact NP probe from a phosphate detectable moiety, said method comprising:

a) providing a sample comprising an intact NP probe with a detectable moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe to incorporate said NP probe on a primer strand hybridized to a target nucleic acid, **[which produces]** a phosphate detectable moiety is produced, wherein said phosphate detectable moiety carries a molecular charge which is different than the molecular charge of said intact NP probe; and

b) applying an energy field to said sample, thereby separating said phosphate detectable moiety from said intact NP probe.

6. (Amended) The method according to claim 5 **[1]**, wherein said polymerase is immobilized.

18. (Amended) A method for identifying an intact charge-switch nucleotide phosphate (NP) probe, said method comprising:

a) contacting a sample comprising an intact charge-switch NP probe having a charged moiety on the base, with an enzyme to produce a phosphate detectable moiety; and

b) applying an electric field to said sample, wherein said phosphate detectable moiety migrates to an electrode differently than said intact charge-switch NP probe.

PENDING CLAIMS

1. (Amended) A method for separating an intact NP probe from a phosphate detectable moiety, said method comprising:

a) providing a sample comprising an intact NP probe with a detectable moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe to incorporate said NP probe on a primer strand hybridized to a target nucleic acid, a phosphate detectable moiety is produced, wherein said phosphate detectable moiety carries a molecular charge which is different than the molecular charge of said intact NP probe; and

b) applying an energy field to said sample, thereby separating said phosphate detectable moiety from said intact NP probe.

2. The method according to claim **1**, wherein said intact NP probe is a charge-switch nucleotide phosphate probe having a detectable moiety on a terminal phosphate.

3. The method according to claim **2**, wherein said charge-switch nucleotide phosphate is a nucleotide triphosphate (NTP) having a γ-phosphate with a detectable moiety attached thereto.

4. The method according to claim **3**, wherein said γ-phosphate with a detectable moiety attached thereto is a γ-phosphate with a fluorophore attached thereto.

5. The method according to claim **1**, wherein said intact NP probe is incorporated on a primer strand hybridized to a target nucleic acid using a polymerase, thereby releasing said phosphate detectable moiety.

6. (Amended) The method according to claim **5**, wherein said polymerase is immobilized.

7. The method according to claim **1**, wherein said energy field is an electric field.

8. The method according to claim 7, wherein said electric field is a first electric field applied in a transverse direction and a second energy field is applied in an axial direction.

9. The method according to claim 8, wherein said second energy field applied in said axial direction is a pressure field.

10. The method according to claim 1, wherein the charge of said phosphate detectable moiety is greater than said intact NP probe.

11. The method according to claim 1, wherein the charge of said phosphate detectable moiety is less than said intact NP probe.

12. The method according to claim 1, wherein the charge of said phosphate detectable moiety is opposite in sign compared to said intact NP probe.

13. The method according to claim 1, further comprising c) detecting said phosphate detectable moiety.

14. The method according to claim 13, wherein said detection is via a charge coupled device (CCD) camera.

15. Canceled.

16. The method according to claim 13, wherein said detection is via a photodiode.

17. The method according to claim 13, wherein said detection is via a blockade current.

18. (Amended) A method for identifying an intact charge-switch nucleotide phosphate (NP) probe, said method comprising:

a) contacting a sample comprising said intact charge-switch NP probe having a charged moiety on the base, with an enzyme to produce a phosphate detectable moiety; and

b) applying an electric field to said sample, wherein said phosphate detectable moiety migrates to an electrode differently than said intact charge-switch NP probe.

19. The method according to claim **18**, wherein said electrode is an anode.

20. The method according to claim **18**, wherein said electrode is a cathode.

21. The method according to claim **18**, wherein either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said phosphate detectable moiety, said phosphate detectable moiety carries a positive charge relative to said intact NP probe.

22. The method according to claim **18**, wherein said enzyme is selected from the group consisting of a DNA polymerase, a DNA dependent RNA polymerase, a reverse transcriptase, a phosphodiesterase and a phosphatase.

23. The method according to claim **18**, wherein said intact charge-switch NP probe is a member selected from the group consisting of a nucleotide diphosphate, a deoxynucleotide triphosphate (dNTP), and a nucleotide triphosphate (NTP).

24. The method according to claim **23**, wherein said deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate deoxythymidine triphosphate and deoxyuridine triphosphate.

25. The method according to claim **18**, wherein said phosphate detectable moiety is a pyrophosphate with a fluorophore moiety attached thereto.

26. The method according to claim **25**, wherein upon cleavage of said pyrophosphate fluorophore moiety, said pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP probe.

27. The method according to claim **18**, wherein said intact NP probe has a positive charge.

28. The method according to claim **18**, wherein said intact NP probe has a negative charge.

29. An intact charge-switch nucleotide phosphate (NP) probe, wherein, upon enzymatic cleavage of said intact charge-switch NP probe to produce a phosphate detectable moiety, said phosphate detectable moiety migrates to an electrode, and intact charge-switch NP probe migrates to the other electrode.

30. The intact charge-switch NP probe according to claim **29**, wherein either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said phosphate detectable moiety, said phosphate detectable moiety carries a molecular positive charge relative to said intact NP probe.

31. The intact charge-switch NP probe according to claim **29**, wherein said charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said phosphate detectable moiety is a pyrophosphate with a fluorophore moiety attached thereto.

32. The intact charge-switch NP probe according to claim **29**, wherein said intact NTP probe has a positive charge.

33. The intact charge-switch NP probe according to claim **31**, wherein upon cleavage of said phosphate detectable moiety as a pyrophosphate fluorophore moiety, said pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP probe.

34. The intact charge-switch NP probe according to claim **29**, wherein said NTP probe is a member selected from the group consisting of a deoxynucleotide triphosphate (dNTP), and a nucleotide triphosphate (NTP).

35. The intact charge-switch NP probe according to claim **34**, wherein said NTP probe is a deoxynucleotide triphosphate (dNTP).

36. The intact charge-switch NP probe according to claim **35**, wherein said deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of

deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate deoxythymidine triphosphate and deoxyuridine triphosphate.

37. The intact charge-switch NP probe according to claim **34**, wherein said nucleotide triphosphate (NTP) is a member selected from the group consisting of adenosine triphosphate, cytosine triphosphate, guanosine triphosphate and uridine triphosphate.

38. The intact charge-switch NP probe according to claim **31**, wherein said fluorophore moiety is attached to said terminal phosphate via a linker.

39. The intact charge-switch NP probe according to claim **38**, wherein said fluorophore linker is an alkylene group having between about 5 to about 12 carbons.

40. The intact charge-switch NP probe according to claim **38**, wherein said linker carries at least one positive charge.

41. The intact charge-switch NP probe according to claim **38** wherein said linker carries at least two positive charges.

42. The intact charge-switch NP probe according to claim **29**, wherein at least one of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen atom with a counter-cation associated therewith.

43. The intact charge-switch NP probe according to claim **29**, wherein said counter-cation is a metal ion.

44. The intact charge-switch NP probe according to claim **43**, wherein said metal ion is selected from the group consisting of Mg^{++}, Mn^{++}, K^{+} and Na^{+}.

45. A method for sequencing a nucleic acid, said method comprising:

providing a target nucleic acid, a primer strand, a polymerase, and a plurality of NP probes;

mixing said target nucleic acid, said sequencing primer, said polymerase, said plurality of NP probes in a flowcell under conditions permitting target dependent polymerization of said plurality of NP probes, thereby providing a polymerization product; and

separating the polymerization products by an energy field in said flowcell to provide a sequence of said target nucleic acid.

46. The method according to claim **45**, wherein the polymerization of said plurality NP probes produces a plurality of phosphate detectable moieties.

47. The method according to claim **45**, wherein said plurality of NP probes are incorporated on said primer strand hybridized to said target nucleic acid using said polymerase, thereby releasing a γ-phosphate with a detectable moiety attached thereto.

48. The method according to claim **45**, wherein said energy field is an electric field.

49. The method according to claim **48**, wherein said electric field is a first electric field applied in the transverse direction and a second electric field applied in the axial direction.

50. A method for sequencing a nucleic acid, said method comprising:

providing a target nucleic acid, a polymerase priming moiety, a polymerase, and a plurality of intact NP probes;

mixing said target nucleic acid, said polymerase priming moiety, said polymerase and said plurality of NP probes under conditions permitting target dependent polymerization of said plurality of NP probes, such conditions which are capable of providing a time sequence of a plurality of phosphate detectable moieties;

separating by charge said plurality of phosphate detectable moieties from said plurality of intact NP probes; and

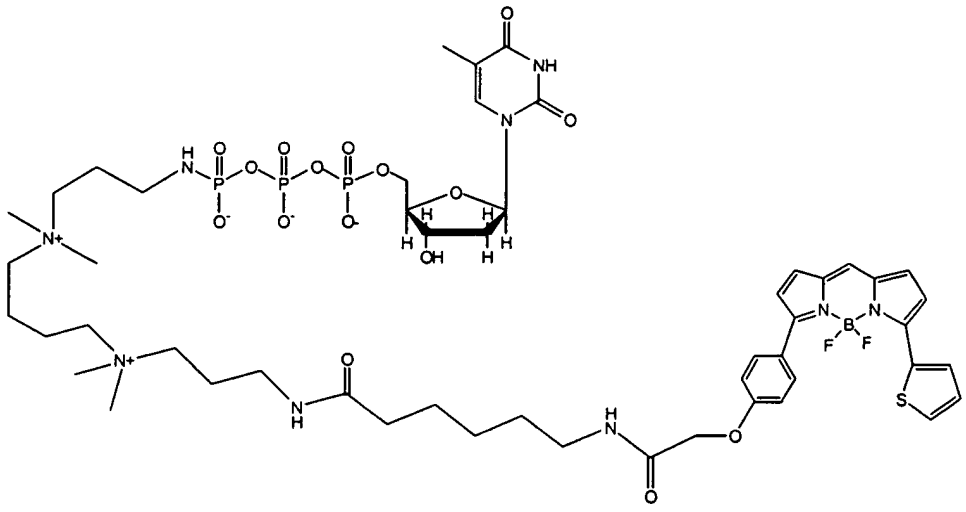
detecting over time said plurality of phosphate detectable moieties to provide a sequence of said target nucleic acid.

51. The method according to claim **50**, wherein said primer moiety is a hairpin loop.

52. The method according to claim **50**, wherein said plurality of phosphate detectable moieties independently selected from the group consisting of PPi-Dye, a terminal phosphate fluorophore moiety, a detectable moiety, charged groups, electrically active groups, reporter groups, and combinations thereof.

53. The method according to claim **52**, wherein said phosphate fluorophore moiety is a used for a member selected from the group consisting of one-color sequencing, two-color sequencing, three-color sequencing, four-color sequencing and combinations thereof.

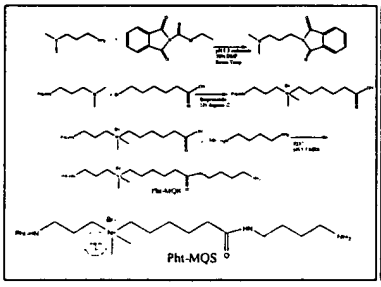
54. The method according to claim **50**, wherein said polymerase is immobilized in single molecule configuration.



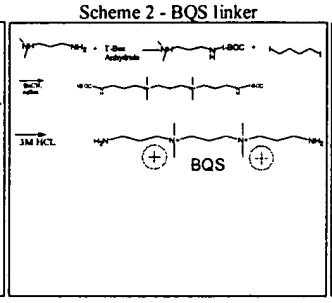
~~dTTP-BQS-BTR~~

FIG. 4

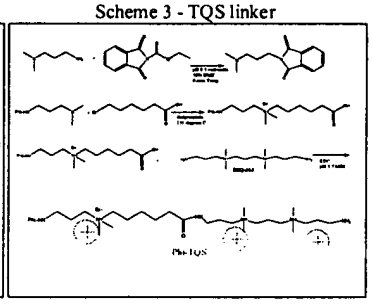
Scheme 1 - MQS component



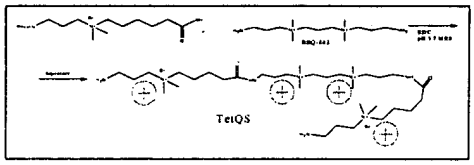
Scheme 2 - BQS linker



Scheme 3 - TQS linker

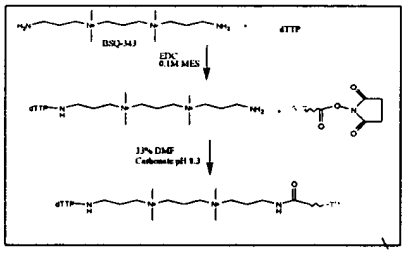


Scheme 4 - TetQS linker



Scheme 5- Protect AA-dUTP

Use same chemistry as in Scheme 1, except the amine is the aminoallyl group of AA-dUTP. ... deprotection can be accomplished in 1M NaOH, room temp, 2 hours, without degrading triphosphates.

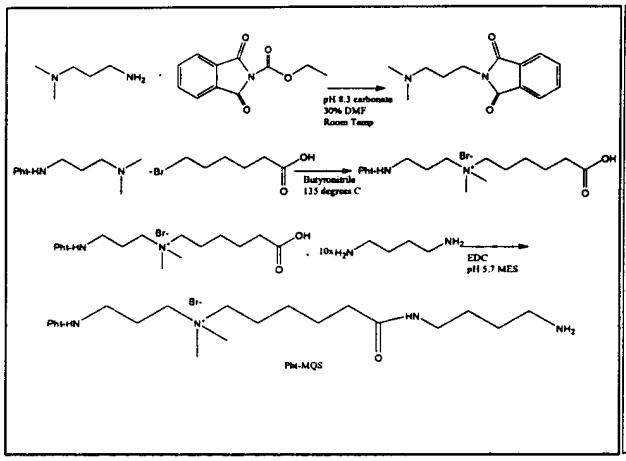


Scheme 6 - Coupling nucleotide, linker and dye (BTR is BodipyTR dye shown as the succinimide ester). ~~We use this chemistry routinely to make γ-dNTPs (e.g. see opd of Fig 13A)).~~

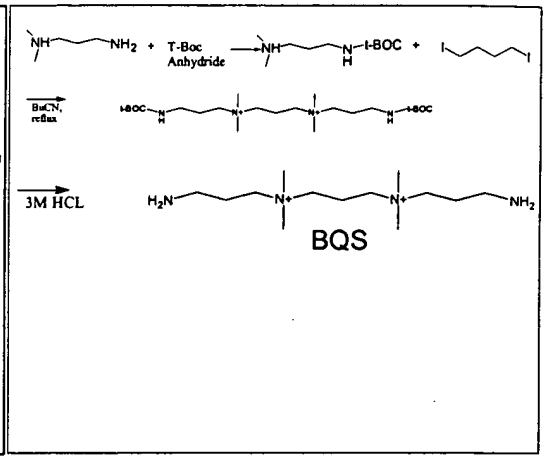
FIG. 6E

10/18

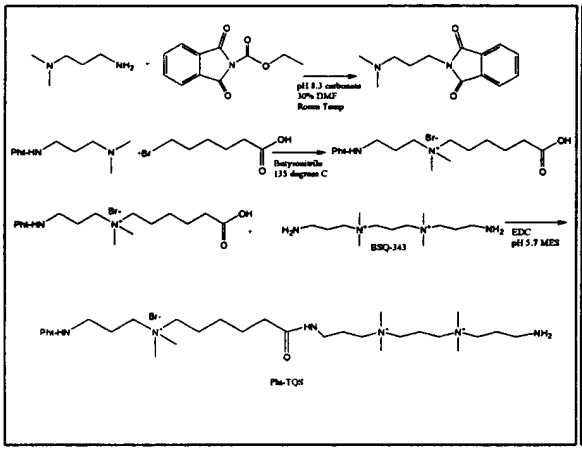
Scheme 1 - MQS component



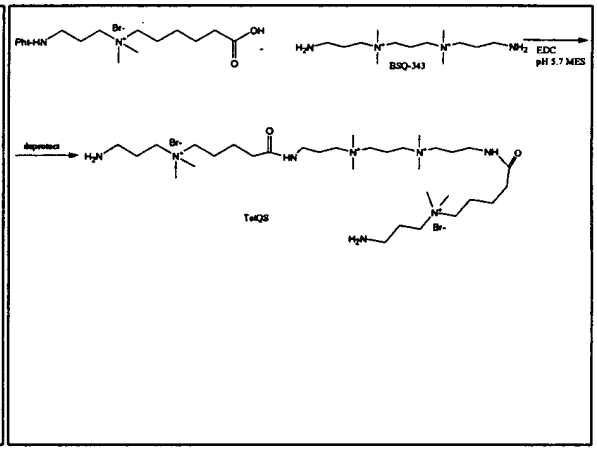
Scheme 2 - BQS linker



Scheme 3 - TQS linker



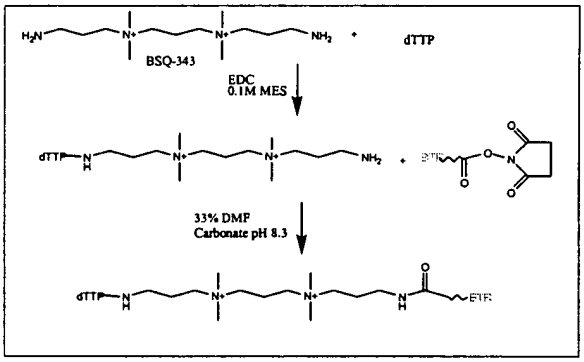
Scheme 4 - TetQS linker



Scheme 5 - Protect AA-dUTP

Use same chemistry as in Scheme 1, except the amine is the aminoallyl group of AA-dUTP. We have shown that deprotection can be accomplished in 1M NaOH, room temp, 2 hours, without degrading triphosphates.

Scheme 6



Coupling nucleotide, linker and dye (BTR is BodipyTR dye shown as the succinimide ester). ~~We use this chemistry routinely to make γ-dNTPs (e.g. see end of Fig 12A).~~

Proposed

FIG. 6E

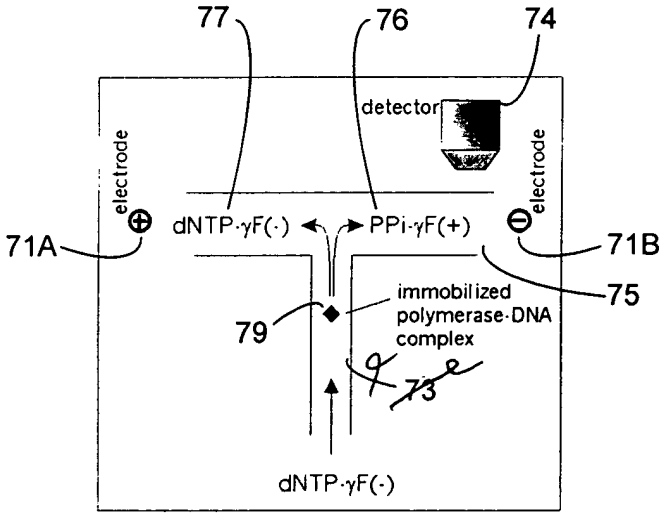


FIG. 7

1000

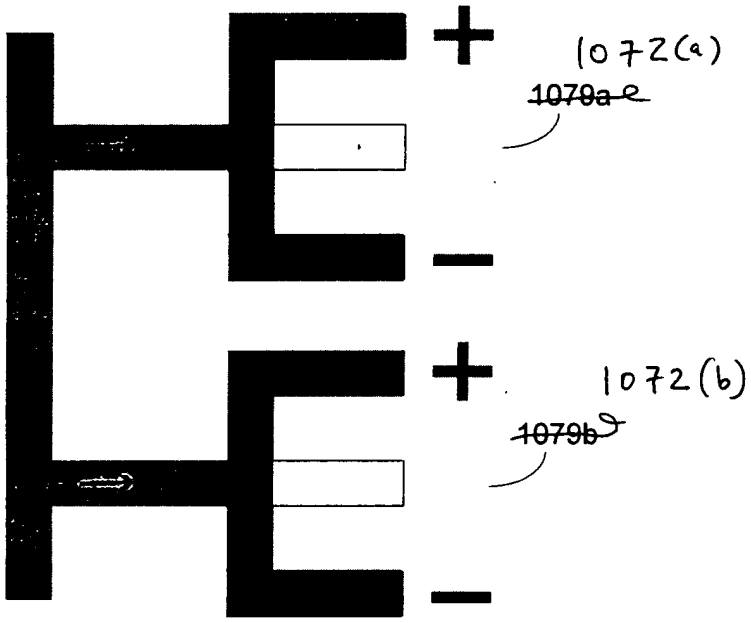


FIG. 10